Influence of Chemical Exposure on Growth of Atlantic Salmon Smolts: Time Series Models Approach

M. Khots, K. Haya, L.E. Burridge, S.B. Brown, W.L. Fairchild

Fisheries and Oceans Canada,
St. Andrews Biological Station, 531 Brandy Cove Road,
St. Andrews, New Brunswick,
E5B 2L9, Canada

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by

M. Khots, K. Haya, L. E. Burridge, S. B. Brown[1], W.L. Fairchild[2]

Fisheries and Oceans Canada, Science Branch, Maritimes Region, St. Andrews Biological Station, 531 Brandy Cove Road, St. Andrews, New Brunswick, E5B 2L9, Canada
[1] Environment Canada, National Water Research Institute, PO Box 5050, Burlington, Ontario, L7R 4A6, Canada
[2] Fisheries and Oceans Canada, PO Box 5030, 343 Université Ave., Moncton, New Brunswick, E1C 9B6, Canada

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ABSTRACT

Khots, M., Haya K., Burridge L.E., Brown S.B.and Fairchild W.L. 2011. Influence of chemical exposure on growth of Atlantic Salmon smolts: time series models approach. Can. Tech. Rep. Fish. Aquat. Sci. 2973: iv +17p.

The change in length and weight of Atlantic salmon (*Salmo salar)* with time was studied in nine groups of fish in a laboratory setting. Atlantic salmon smolts were exposed, in freshwater, to either 17-β-estradiol (E2) or 4-nonylphenol (4-NP). Exposures took place at three different times in May and June of 1999. The fish were subsequently transferred to seawater and their growth was monitored over a four-month period. The purpose was to determine if these compounds affect growth of Atlantic salmon smolts and if the timing of exposure has an effect on the response. In this article we describe the effects of chemical treatment on growth. Using weighted regression and comparison of mathematical models, we showed that short term exposure of smolts to E2 and 4-NP significantly reduces the rate of growth of salmon smolts in seawater.

RÉSUMÉ

Khots, M., Haya K., Burridge L.E., Brown S.B.and Fairchild W.L. 2011. Influence of chemical exposure on growth of Atlantic Salmon smolts: time series models approach. Can. Tech. Rep. Fish. Aquat. Sci. 2973: iv +17p.

La modification au fil du temps de la longueur et du poids du saumon de l'Atlantique (*Salmo salar*) a été étudiée sur neuf groupes de poissons dans un laboratoire. De jeunes saumons de l'Atlantique ont été exposés, en eau douce, à du 17 β-estradiol (E2) ou du 4-nonylphénol (4-NP). L'exposition a eu lieu à trois différents moments, en mai et en juin 1999. Les poissons étaient par la suite transférés en eau salée et leur croissance était suivie sur une période de quatre mois. L'objectif était de déterminer si ces composés ont un effet sur la croissance des jeunes saumons de l'Atlantique et si le moment de l'exposition influence la réaction. Dans le présent article, nous décrivons les effets du traitement chimique sur la croissance. À l'aide d'une régression pondérée et d'une comparaison des modèles mathématiques, nous avons démontré qu'une exposition à court terme des saumoneaux au E2 et au 4-NP réduit considérablement le taux de croissance des saumoneaux en eau salée.

INTRODUCTION

Indigenous populations of Atlantic salmon (*Salmo salar* L.) have been decreasing throughout the northwest Atlantic area for a number of years (Fairchild *et al.* 1999). The cause of this decline remains unclear. Atlantic salmon hatch in freshwater streams, spend several years as juveniles then move downstream to the North Atlantic Ocean. The physiological change required for the fish to survive in seawater is called smoltification or parr to smolt transformation (PST) and salmon undergoing this change are called smolts (McCormick and Saunders, 1987, McCormick *et al.* 1998). Carey and McCormick, 1998 have shown that this is a sensitive life stage for salmon as they show a greater biochemical stress response than juvenile fish exposed to the same stressor (handling and confinement). Hontela (1997) states that fish show a wide range of responses to xenobiotics. In some cases the chemicals elicit a typical stress response in other cases the contaminant affects the fish's ability to respond to the stressor.

There is evidence that smolts moving downstream may be exposed to effluent from wastewater treatment (industrial or municipal). Some of the chemical constituents of these effluents may be able to cause effects in whole organisms, progeny, or populations via actions on the endocrine system, and are known as endocrine disrupting substances (EDSs) (Servos,1999).

One of the compounds that fish may be exposed to is the female hormone, estrogen, 17 β-estradiol (E2). Estrogen is released in effluent from municipal waste water treatment plants often into rivers and estuaries frequented by migrating salmon. Arsenault *et al.* 2004 reported that this chemical affects growth in Atlantic salmon and discussed possible mechanisms of this effect. Kidd *et al.* 2007, found that long term exposure of fathead minnows to E2 resulted in substantial changes in a small lake of Canada. They showed that chemical treatment leads to a collapse of the fish population and to a relative decrease of the rate of growth characteristics in comparison to fish without chemical treatment.

Fairchild *et al.* 1999, studied the relationship between the potential exposure of Atlantic salmon smolts to 4-Nonylphenol (4-NP) and the return of adult salmon to rivers of New Brunswick, Canada. This chemical is a breakdown product of nonylphenol ethoxylates (NPEs). NPEs are constituents of industrial and domestic cleaning products, petroleum products and wastewater from pulp and paper industries and textile manufacturing facilities. 4-NP is commonly found in discharges from sewage treatment plants and in industrial effluents (Madsen *et al.* 1997; Naylor *et al.* 1998). 4-NP also has been shown to act as an estrogen mimic in fish (Madsen *et al.* 1997).

In this article we report results of a study conducted in 1999 to investigate the effects of E2 and 4-NP on growth characteristics (length and weight) of Atlantic salmon smolts with time. We describe smolt growth after transfer of fish to seawater and effects of exposure to chemical contaminants. Our purpose in conducting this study was to determine if exposure to chemicals during PST affected subsequent performance in seawater.

To help assess the effects of chemical treatment on the growth of smolts, we used the results of our research (Khots *et al.* 2010) describing the growth of fish in fresh and seawater without chemical treatment. Previous studies have described statistical methods

for assessing fish growth (Millar 2004, Newman 2000, Beckman 2004). These studies have focused on wild stocks or stocks of salmon being held and raised for aquaculture purposes.
We present a new statistical approach consisting of several parts: preliminary data processing, development, validation and comparison of mathematical models of the growth characteristics of Atlantic salmon smolts with and without chemical treatment.

Materials and methods

In January 1999, fourteen-month post-hatch Atlantic salmon parr were obtained from the Huntsman Marine Science Centre Chamcook Hatchery, St. Andrews, NB, Canada, and transferred to the St. Andrews Biological Station. Parr (75-80 g) were anesthetized in 1% tert-amyl alcohol and individually tagged with passive integrated transponder (PIT) tags (Biomark, Boise, Idaho). Fish were randomly distributed into 16 fiberglass tanks (400 L, n=50 per tank) and allowed to acclimate in dechlorinated St. Andrews, NB, municipal water at ambient temperature for three months prior to treatments. The tanks were covered and enclosed in individual compartments, each with their own water supply and light. The flow rate was maintained at approximately 5L/min. Photoperiod was regulated to simulate natural photoperiod. Except on treatment and sampling days, the fish were fed by hand twice daily to satiation with a premium quality open formula diet (Moore-Clark, a Division of Nutreco Canada Inc., St. Andrews, NB).
In May, the juvenile salmon were exposed to water-borne 4-NP and E2 on three different occasions {Early Window (May 12-16), Middle Window (May 26-30), Late Window (June 9-13)} during the PST period. Two replicate tanks were treated with an environmentally-relevant concentration of 4-NP (20 μg/L) (Fairchild *et al.* 1999) and with E2 (100 ng/L), serving as a positive control.
The test substances were dissolved in ethanol and diluted with water such that ethanol represented 10% of the delivery solution. Control tanks received the 10% ethanol vehicle. 4-NP was delivered in two 24-hour pulses (day 1 and day 6) at a flow rate of 1 mL/min using a Mariott bottle system. E2 was delivered continuously throughout the treatment. Beginning 12-14 days after the onset of the treatment at each time, fish were gradually acclimated to filtered sea water over a five-day period. The flow was maintained at approximately 5L/min. In June, July and October a sub-sample of each treatment group was sacrificed for biochemical analysis and the remaining fish were anaesthetized and their length and weight were recorded. The sampling procedure took 2 days, one day for biochemistry and one for length/weight determination. The accuracy of measurements is 0.1 centimeter (cm) for length, 0.1 gram (g) for weight and 1 day for time. At the end of August 1999 the fish were moved to two large tanks and held until the final sampling in October.

Statistical Method Development

To evaluate the effect of chemicals on the growth of salmon smolts, we considered the data (separately for each chemical) regardless of the starting date of treatment. Statistical packages STATA and MS EXCEL were used to perform calculations and to draw figures.

Table 1: Number of length/weight measurements for each treatment at sampling times ranging from 160 to 289 days after the first measurements (January 5, 6 and 7, 1999). Number of measurements in validation set for each coordinate is written in brackets.

Coordinate Number	Day	Number of measurements for:	
		E2 Treatment	NP Treatment
1	160	2	20
2	161	24 (8)	24 (8)
3	162	16	8
4	195	8 (8)	32
5	196	50(17)	49 (8)
6	197	29	16
7	202	0	35
8	203	35	15 (15)
9	230	0	35
10	231	51 (19)	50 (14)
11	232	24	18
12	279	0	16
13	280	26 (9)	24 (7)
14	281	16	9
15	287	0	20
16	288	28 (10)	25 (8)
17	289	0	6

Processing of data included three parts:
Part 1. Preliminary processing of data (was performed according to Khots et al. 2010).
Pat 2. Development and validation of mathematical models for the study of dependences Time – Length and Time – Weight for smolts under chemical treatment.
Part 3. Comparison of models obtained on the basis of observations of fish with and without chemical treatment.
Development and validation of mathematical models was performed for length and weight observations independently. It included:

First, we used the mean values of length and weight for each of the coordinates of time vector (results of preliminary data processing). Since these mean values had different standard deviations, the statistical weights (RW) of coordinates were obtained.
RW is calculated in three steps:

- Estimation of the standard deviations of measurements (SD_i);
- Division of SD_i by the square root of the number of measurements to calculate SD_i(mean);
- Computation of RW as inverse values of SD_i(mean).

After obtaining RW for each point, we used the method of weighted least squares (Draper and Smith. 1998) to approximate the dependences Time - Length by polynomials of the first order and Time - Weight by polynomials of the second order. To test these models statistically, we applied F-test.
We denoted functions under consideration as $L_i(t)$ for dependences Time – Length and $W_i(t)$ for dependences Time – Weight

(1) $L_i(t) = a_{0i} + a_{1i}*t,$ $i= 1, 2$

(2) $W_i(t) = b_{0i} + b_{1i}*t + b_{2i}*t\text{^}2,$ $i= 1, 2$

i corresponds to type of chemical treatment, specifically $i = 1$ for E2 treatment and $i = 2$ for 4-NP treatment.
The scaled variable t was defined by formula:
$t = (T-160)/124$
where variable T is time (number of days) from PIT tagging in January 1999 (Table 1); $T = 160$ (days) is the beginning of time segment under study; 124 (days) is the length of the time from the first post-treatment sampling (June) until the final post-treatment sampling (October).
To validate the developed models, we applied the Holdout method (Kriek *et al.* 2007). In accordance with this method the initial data is randomly separated into two parts: validation set (which was less than a third of the initial sample) and training set; the sets do not intersect. Mathematical models are created for the training set and tested on the validation set.
In our case, we performed the following successive steps:
- Selection of validation and training sets (data from one of the tanks with E2 treatment and one of the tanks with 4-NP treatment were considered as validation sets; data from four tanks with E2 treatment and data from five tanks with 4-NP treatment were considered as training sets);
- Construction of mathematical models $L_{i,trng}(t)$ and $W_{i,trng}(t)$, where $i = 1, 2$, on the basis of training sets;
- Testing of developed mathematical models by F-test;
- Comparison of experimental data in validation sets with results obtained by mathematical models; we determined $F_{obs\ val}(L_{i,trng}(t))$ and $F_{obs\ val}(W_{i,trng}(t))$, $i = 1, 2$, as the sums of squares of differences between experimental and calculated values divided by the number of degrees of freedom;
- Comparison of $F_{obs\ val}(L_{i,trng}(t))$ and $F_{obs\ val}(W_{i,trng}(t))$ with $F_{crit}(0.95, s, \infty)$, where 0.95 is confidence level, and s is number of degrees of freedom;
- Creation of mathematical models based on united training and validation sets.

To estimate the dispersion of functions $L_i(t)$ and $W_i(t)$, $i = 1, 2$, we applied the confidence band technique (Draper and Smith. 1998). For simplicity, we denote functions $L_i(t)$ and $W_i(t)$, $i = 1, 2$ as G(t). The lower boundary (LB) and upper boundary (UB) of these functions are:

(3) $LB(G(t)) = G(t) - 1.96*(Var(G(t))\hat{}0.5$

(4) $UB(G(t)) = G(t) + 1.96*(Var(G(t))\hat{}0.5$

where

$Var(L_i(t)) = Var(a_{0i}) + 2*Cov(a_{0i}, a_{1i})*t + Var(a_{1i})*t\hat{}2$

$Var(W_i(t)) = Var(b_{0i}) + 2*Cov(b_{0i}, b_{1i})*t + [Var(b_{1i}) + 2*Cov(b_{0i}, b_{2i})]*t\hat{}2 + 2*Cov(b_{1i}, b_{2i})*t\hat{}3 + Var(b_{2i})*t\hat{}4$

The number 1.96 in (3) and (4) corresponds to confidence level $p = 0.95$.
To evaluate the rate of the weight change, we use the derivatives $W'_i(t)$ and $Var(W'_i(t))$, where $i = 1, 2$:

$W'_i(t) = b_{1i} + 2*b_{2i}*t$

$Var(W'_i(t)) = Var(b_{1i}) + 4*Cov(b_{1i}, b_{2i})*t + 4*Var(b_{2i})*t\hat{}2$

To determine the influence of chemicals on the length and weight change, we used the equations for smolts growth without chemical treatment: Dependence Time - Length L(t), Dependence Time – Weight W(t), and their variances Var(L(t)) and Var(W(t)), (Khots *et*

al. 2010). Below we denote coefficients of L(t) as a_0 and a_1 and coefficients of W(t) as b_0, b_1 and b_2.
We studied separately three sets of mathematical models:
$\{L(t), L_1(t), L_2(t)\}$, $\{W(t), W_1(t), W_2(t)\}$, $\{W'(t), W'_1(t), W'_2(t)\}$
For each of these sets we compared all possible pairs of models.
For simplicity we use $G_1(t)$ and $G_2(t)$ to denote two compared functions. To compare two models, we performed the following successive steps.
a) Determination of $LB(G_i(t))$ and $UB(G_i(t))$, $i = 1, 2$;
b) Solution of equations

(5) $$G_1(t) - LB(G_2(t)) = 0$$
(6) $$G_1(t) - UB(G_2(t)) = 0$$
and/or
(7) $$G_2(t) - LB(G_1(t)) = 0$$
(8) $$G_2(t) - UB(G_1(t)) = 0$$

c) Split of time segment under consideration into time sub-segments
(9) V(-), V(0), V(+), and V(U)
defined as

- V(-): $G_1(t)$ is statistically less than $G_2(t)$ for any $t \in V(-)$;
- V(0): $G_1(t)$ is not statistically different from $G_2(t)$ for any $t \in V(0)$;
- V(+): $G_1(t)$ is statistically more than $G_2(t)$ for any $t \in V(+)$;
- V(U): uncertain statistical relation between $G_1(t)$ and $G_2(t)$, i.e. confidence band of $G_1(t)$ covers $G_2(t)$, and confidence band of $G_2(t)$ does not cover $G_1(t)$, for any $t \in V(U)$, or vice versa, confidence band of $G_1(t)$ does not cover $G_2(t)$, and confidence band of $G_2(t)$ covers $G_1(t)$, for any $t \in V(U)$.

The sub-segments V(U) are located usually between time sub-segments V(-) and V(0) or between V(0) and V(+).
In principle, we could transform irrational equations (5) through (8) into their algebraic form to calculate the roots and determine the sub-segments. In this case, we would obtain algebraic equations of the second order when we compare the three pairs of models for length and the three pairs of models for the derivatives of weight; these equations would each have two complex roots. For the three pairs of models for weight, algebraic equations of the fourth order with four complex roots could be calculated – a rather tedious process since it includes successive application of Cardano's formula (solution of algebraic equation of the third order) and Ferrari scheme (Kurosh 1968). Meanwhile, we do not need to estimate all possible complex roots of equations (5) through (8) because
- the transformed equations can have the additional roots in comparison with original equations;
- it is not clear a priori how many roots of equations are located in the time segment under study;
- the acceptable accuracy of roots is limited by accuracy in the time scale of experiment.

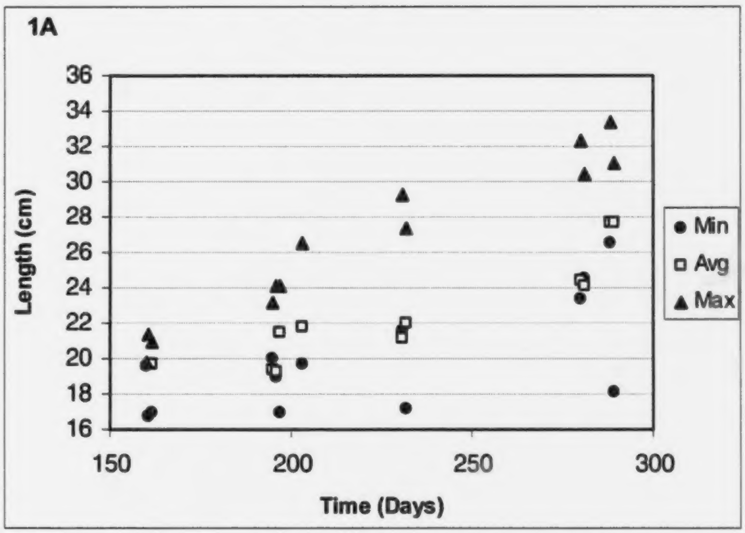
The suggested procedure in this article for the calculation of roots is based on the method of successive approximation, containing three steps:
- computation of left sides of equations (5) through (8) with known accuracy in time scale;
- selection of adjacent time points with negative and positive values of functions;

- linear interpolation of functions between these adjacent points to approximate roots.

Results and discussion

Our statistical approach allowed us to study complex biological process of growth of fish under chemical treatment in detail.
Figures 1A, 1B, 2A, and 2B respectively show the change in length and weight of salmon under E2 and 4-NP treatments from June through October.
As can be seen in these Figures, Atlantic salmon exposed to E2 or 4-NP can grow to a maximum length of 34-36 centimetres and maximum weight of 380-500 grams, during the study. Meanwhile the difference between maximum and minimum values of the growth characteristics can vary in wide boundaries:

- For fish under E2 treatment (maximum of length – minimum of length)/(average of length) is changed from 0.01 to 0.48, and (maximum of weight – minimum of weight)/(average of weight) is changed from 0.03 to 1.05
- For fish under 4-NP treatment (maximum of length – minimum of length)/(average of length) is changed from 0.05 to 0.39, and (maximum of weight – minimum of weight)/(average of weight) is changed from 0.14 to 1.44.



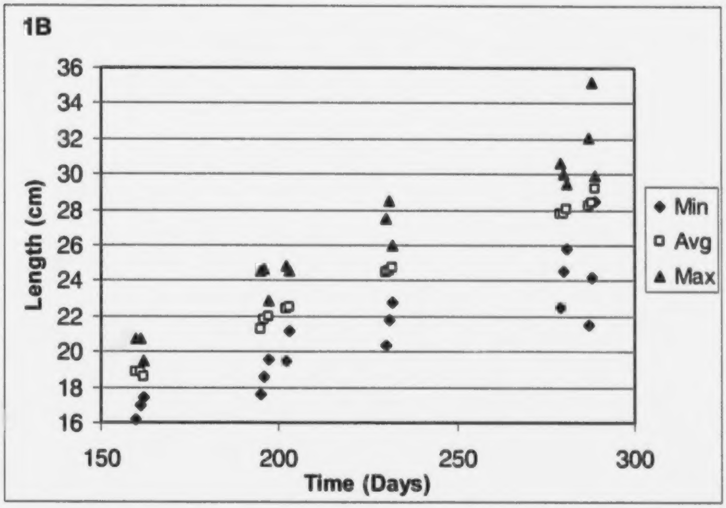
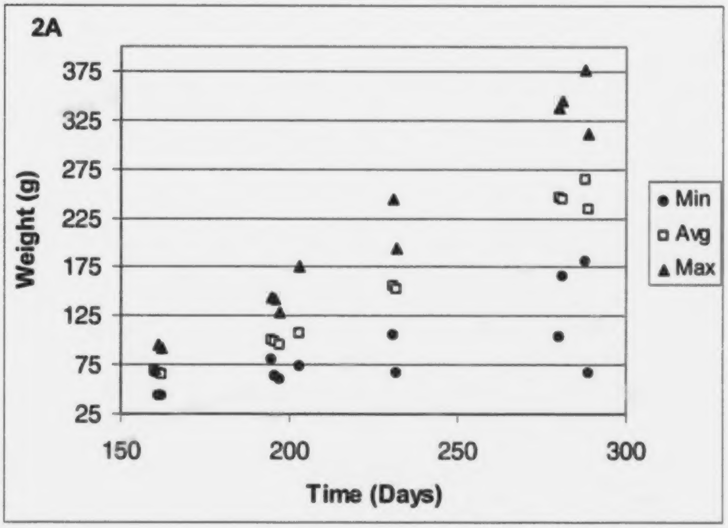


Figure 1.Growth of Atlantic salmon length in seawater subsequent to exposure to either E2 (**1A**) or 4-NP (**1B**)



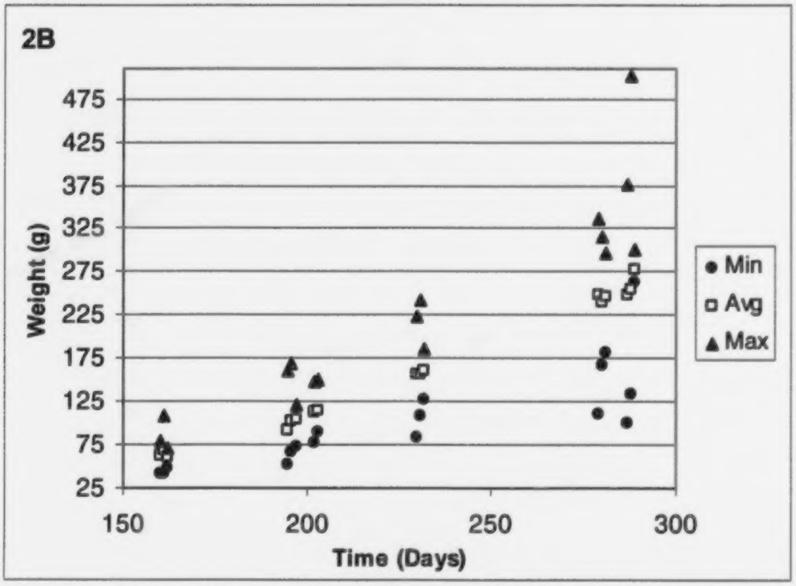


Figure 2. Growth of Atlantic salmon weight in seawater subsequent to exposure to either E2 (**2A**) or 4-NP (**2B**)

We used the corresponding regression equations and their statistical characteristics (Tables 2 and 3).

Table 2. Validation of models Time – Length and Time – Weight
L1,trng(t) model Time - Length of smolts under E2 treatment on training set;
L2,trng(t) model Time - Length of smolts under 4-NP treatment on training set;
W1,trng(t) model Time – Weight of smolts under E2 treatment on training set;
W2,trng(t) model Time – Weight of smolts under 4-NP treatment on training set.

Models	Constant Term	Coefficient of the Term of the First Order	Coefficient of the Term of the Second Order	Fobs trng	$F_{crit}(0.95, m, \infty)$	Fobs val	$F_{crit}(0.95, s, \infty)$
$L_{1,trng}(t)$	18.96	9.17		2.51	3.00 (m=2)	0.62	1.34
$L_{2,trng}(t)$	19.07	9.24		1.89	3.00 (m=2)	0.64	1.34
$W_{1,trng}(t)$	64.8	89.5	102	3.51	3.84 (m=1)	0.7	1.34
$W_{2,trng}(t)$	63.3	113.9	74.5	1.57	3.84 (m=1)	0.73	1.34

Using obtained results, we conclude that:

- $F_{obs\ val}(L_{i,trng}(t))$ and $F_{obs\ val}(W_{i,trng}(t))$, i=1, 2, were less than $F_{crit}(0.95, s, \infty) = 1.34$ (Table 2). In the validation procedure, the number of degrees of freedom (s) ranged from 55 to 65;
- Observed values $F_{obs}(L_i(t))$, and $F_{obs}(W_i(t))$, i=1, 2, were less than $F_{crit}(0.95, m, \infty)$ for regression equations under study (Tables 2, 3).

Evaluation of the influence of chemical treatment on the growth of smolts was performed successively.

Study of length of smolts

- Both chemicals decrease the progression of length of salmon smolts permanently during the experiment:
    - $L(t)$ was statistically more than $L_1(t)$ for any t ε [0,1];
    - $L(t)$ was statistically more than $L_2(t)$ for any t ε [0,1];
- Effects of E2 and 4-NP treatments were similar: the difference between models $L_1(t)$ and $L_2(t)$ was not statistically significant for any t ε [0,1].

Table 3.Models Time – Length and Time – Weight and their statistical characteristics

L(t) model Time – Length of smolts without chemical treatment;

L1(t) model Time – Length of smolts under E2 treatment;

L2(t) model Time – Length of smolts under 4-NP treatment;

W(t) model Time – Weight of smolts without chemical treatment;

W1(t) model Time – Weight of smolts under E2 treatment;

W2(t) model Time – Weight of smolts under 4-NP treatment.

Models	Constant Term	Coefficient of the Term of the First Order	Coefficient of the Term of the Second Order	Fobs	$F_{crit}(0.95, m, \infty)$
$L_1(t)$	18.99	9.23		1.51	3.00 (m=2)
$L_2(t)$	19.03	9.37		2.02	3.00 (m = 2)
$L(t)$	19.32	9.79		1.9	3.00 (m=2)
$W_1(t)$	64.9	89.7	100.7	3	3.84 (m = 1)
$W_2(t)$	62.8	113.1	76.7	2.55	3.84 (m = 1)
$W(t)$	67.6	83.9	134.7	2.02	3.84 (m = 1)

Models	Var(Constant Term)	2*Cov(Constant Term, Coefficient of the Term of the First Order)	Var(Coefficient of the Term of the First Order)	2*Cov(Constant Term, Coefficient of the Term of the Second Order)	2*Cov(Coefficient of the Term of the First Order, Coefficient of the Term of the Second Order)	Var(Coefficient of the Term of the Second Order)
$L_1(t)$	0.0194	-0.0214	0.0385			
$L_2(t)$	0.0149	-0.0164	0.0295			
$L(t)$	0.0171	-0.0187	0.0268			
$W_1(t)$	3.51	-1.21	22.12	1.65	-67.04	70.66
$W_2(t)$	2.15	-0.68	20.04	1.08	-65.56	72.21
$W(t)$	3.19	-1.17	23.09	1.86	-79.17	87.55

Table 3 shows:
a) Intercept a_0 is statistically more than intercept a_{01} and intercept a_{02};
b) Difference between intercepts a_{01} and a_{02} is not statistically significant;
c) Slope a_1 is statistically more than slope a_{11} and slope a_{12};
d) Difference between slopes a_{11} and a_{12} is not statistically significant.
To show the tendency in the change of mean values of length of salmon smolts under E2 and 4-NP treatment in comparison with mean values of length of salmon smolts without treatment in time, we calculated the ratios $(L(t)-L_1(t))/L(t)$ and $(L(t)-L_2(t))/L(t)$.
Our results indicated that these ratios increased in time from 1.7% to 3.1% (with E2 treatment), and from 1.5% to 2.5% (with 4-NP treatment).

Study of weight of smolts

On the basis of Table 3, we established statistical relations between coefficients of $W(t)$, $W_1(t)$, and $W_2(t)$. These relations are more complicated than for the coefficients of length models.

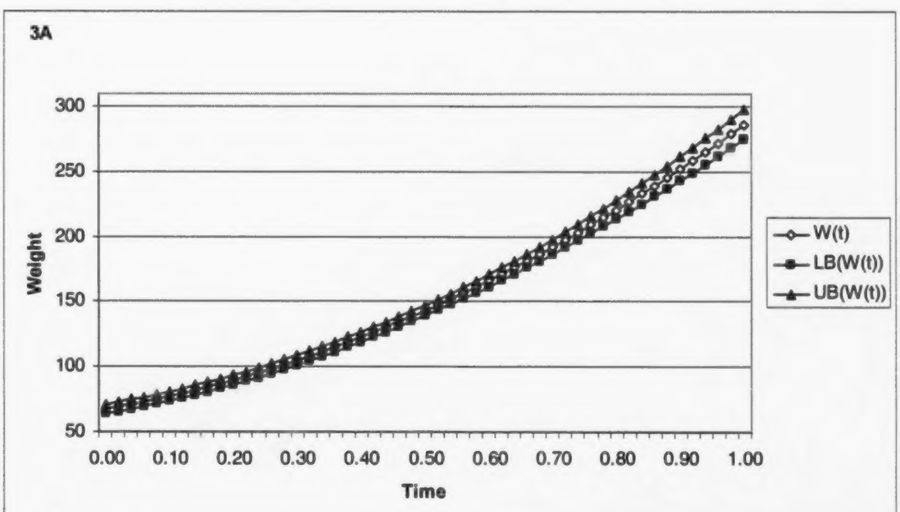
- For constant terms, the differences between b_0 and b_{01} and between b_{01} and b_{02} are not statistically significant; and b_0 is statistically more than b_{02}.
- For coefficients of the terms of the first order, the difference between b_1 and b_{11} is not statistically significant; b_1 is statistically less than b_{12} and b_{11} is statistically less than b_{12}.
- For coefficients of the terms of the second order we have statistically significant differences: $b_{22} < b_{21} < b_2$

These results force us to compare the models on the whole. We applied the technique presented in Part 3 of Statistical Methods Development.

Comparison of weight models

Confidence bands of functions $W(t)$, $W_1(t)$, and $W_2(t)$ are illustrated in Figs 3A, 3B and 3C. The curves in these figures were used for comparison of weight models:

- W(t) has intersections with UB(W1(t)) and with UB(W2(t));
- LB(W(t)) intersects W1(t) and W2(t);
- W1(t) intersects LB(W2(t)).



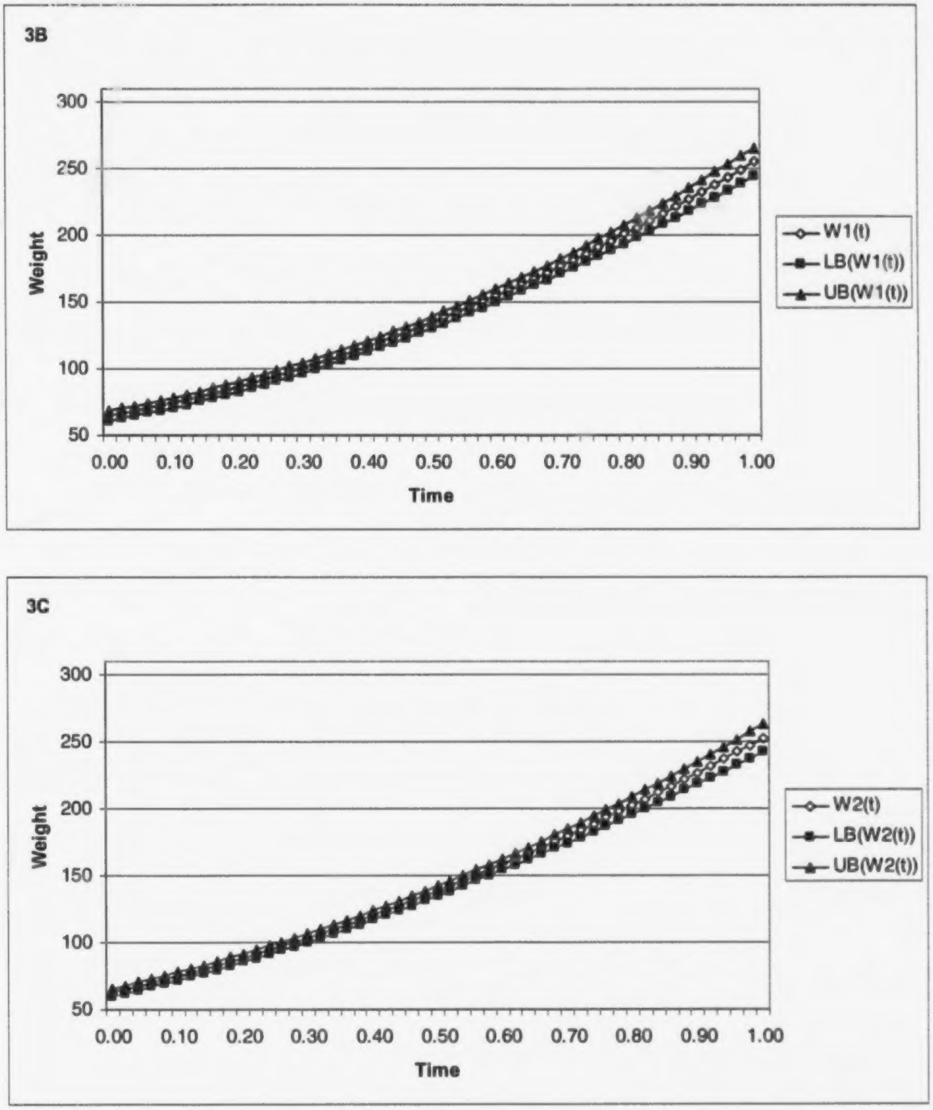


Figure 3. Average weight over the course of the experiment of controls W(t) (**3A**), E2 treated fish $W_1(t)$ (**3B**) and 4-NP treated fish $W_2(t)$ (**3C**), and their upper and lower confidence bands.

The roots of the equations and the split of time segment [0,1] into sub-segments (9) are presented in Table 4. Applying inverse transformation of independent variable t into T (days after beginning of experiment): $T = 124*t + 160$, we show these results in calendar year dates (Table 4). The corresponding time sub-segments are written with letter R.

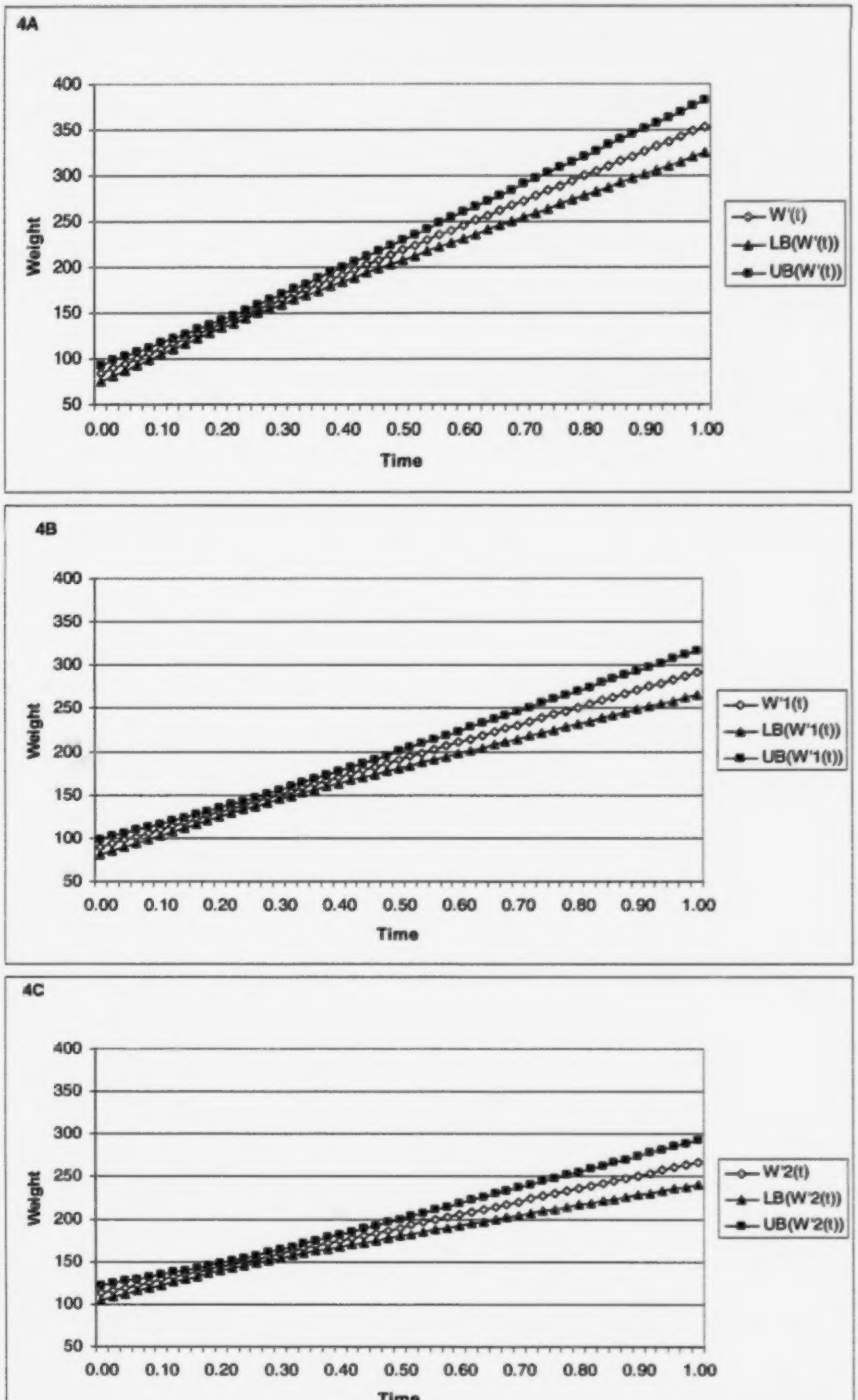


Figure 4. Average rate of weight change over the course of the experiment of controls W'(t) (**4A**), E2 treated fish W'1(t) (**4B**) and 4-NP treated fish W'2(t) (**4C**), and their upper and lower confidence bands.

Pairs of weight models	Equations	Solutions of equations in time segment [0,1]	Sub-segments in time segment [0,1]				Sub-segments in calendar year dates			
			V(-)	V(0)	V(+)	V(U)	$V_R(-)$	$V_R(0)$	$V_R(+)$	$V_R(U)$
$(W(t), W_1(t))$	$W(t) - UB(W_1(t)) = 0$ $W_1(t) - LB(W(t)) = 0$	0.292 0.276		[0,0.276]	[0.292,1]	[0.276,0.292]		[Jun 15,Jul 17]	[Jul 20,Oct 17]	[Jul 18,Jul 19]
$(W(t), W_2(t))$	$W(t) - UB(W_2(t)) = 0$ $W_2(t) - LB(W(t)) = 0$	0.077; 0.453 0.050; 0.477		[0.077,0.453]	[0,0.050] and [0.477,1]	[0.050,0.077] and [0.453,0.477]		[Jun 24,Aug 8]	[Jun 15,Jun 20] and [Aug 12,Oct 17]	[Jun 21,Jun 23] and [Aug 9,Aug 11]
$(W_1(t), W_2(t))$	$W_1(t) - LB(W_2(t)) = 0$	0.362; 0.510		[0,0.362] and [0.510,1]		[0.362,0.51]		[Jun 15,Jul 29] and [Aug 17,Oct 17]		[Jul 30,Aug 16]

Pairs of derivatives of weight models	Equations	Solutions of equations in time segment [0,1]	Sub-segments in time segment [0,1]				Sub-segments in calendar year dates			
			V'(-)	V'(0)	V'(+)	V'(U)	$V'_R(-)$	$V'_R(0)$	$V'_R(+)$	$V'_R(U)$
$(W'(t), W'_1(t))$	$W'(t) - UB(W'_1(t)) = 0$ $W'_1(t) - LB(W'(t)) = 0$	0.165 0.16		[0,0.160]	[0.165,1]	[0.160,0.165]		[Jun 15,Jul 18]	[Jul 20,Oct 17]	[Jul 19]
$(W'(t), W'_2(t))$	$W'(t) - LB(W'_2(t)) = 0$ $W'(t) - UB(W'_2(t)) = 0$ $W'_2(t) - LB(W'(t)) = 0$ $W'_2(t) - UB(W'(t)) = 0$	0.214 0.295 0.297 0.213	[0,0.213]	[0.214,0.295]	[0.297,1]	[0.213,0.214] and [0.295,0.297]	[Jun 15,Jul 11]	[Jul 12,Jul 15]	[Jul 16,Oct 17]	
$(W'_1(t), W'_2(t))$	$W'_1(t) - LB(W'_2(t)) = 0$ $W'_2(t) - UB(W'_1(t)) = 0$	0.36 0.358	[0,0.358]	[0.36,1]		[0.358,0.36]	[Jun 15,Jul 29]	[Jul 30,Oct 17]		

Table 4. The split of time segment for different pairs of mathematical models
W(t) model Time – Weight of smolts without chemical treatment;
W1(t) model Time – Weight of smolts under E2 treatment;
W2(t) model Time – Weight of smolts under 4-NP treatment;
W'(t) model Time – Rate of Weight Change of smolts without chemical treatment;
W'1(t) model Time – Rate of Weight Change of smolts under E2 treatment;
W'2(t) model Time – Rate of Weight Change of smolts under 4-NP treatment.

Comparison of derivatives of weight models

Confidence bands of functions W'(t), $W'_1(t)$ and $W'_2(t)$ are illustrated in Figs 4A, 4B and 4C. We used the curves in these figures for comparison of derivatives of weight models. As can be seen in these Figures, W'(t) has intersections with UB(W'1(t)) and with UB(W'2(t)); line LB(W'(t)) intersects W'1(t) and W'2(t); line W'1(t) intersects LB(W'2(t)), and so on.

The solutions of corresponding equations and the split of time segment [0,1] into sub-segments (9) are given in Table 4. We also showed the results in calendar year dates. Similar to time sub-segments V(-), V(0), V(+), and V(U), we use V'(-), V'(0), V'(+), and V'(U) to compare the derivatives of weight models. In Table 4, $V'_R(U)$ were excluded for pairs of models (W'(t), $W'_2(t)$) and ($W'_1(t)$, $W'_2(t)$) because their lengths were less than half a day (accuracy in time scale of experiment is one day).

Summarizing the results of calculations for weight, we concluded:

- Both E2 and 4-NP treatments decreased the growth of weight of salmon smolts in comparison with growth without treatment. We could detect this phenomenon after certain delay (T_{del}) depending on the type of treatment.
  Weight gain of salmon smolts without treatment was greater than that observed with:
  - E2 treatment after about seven weeks ($T_{del} \approx 7$ weeks);
  - 4-NP treatment after about two months ($T_{del} \approx 2$ months).
- The rate of weight of Atlantic salmon smolts without treatment was higher than the rate of weight under:
  - E2 treatment after about five weeks ($T_{del} \approx 5$ weeks);
  - 4-NP treatment after about seven weeks ($T_{del} \approx 7$ weeks).
- The differences among the treatments are not significant:
  - model of weight growth with E2 treatment was not statistically different from model of weight growth with 4-NP treatment (we observed only small sub-segment of uncertainty in the middle of the experiment);
  - corresponding models for derivatives were statistically different only in the beginning of the experiment ($T_{del} <$ seven weeks);
  - we could observe the difference between E2 and 4-NP treatments on the second derivative of the weight models, i.e. the acceleration of weight growth under E2 treatment was statistically more than the acceleration of weight growth under 4-NP treatment.

These quantitative results were supported by independent biological observations. Several experiments have been conducted investigating the effects of water-borne exposure of smolts to 4-NP and E2 on growth. A consistent result is that a portion of the smolts treated with 4-NP or E2 experienced compromised growth (see for example, Fairchild et al., 2000). The mechanism by which this compound influences growth is unknown. It remains unclear whether the response is chemical specific or whether the effect on growth is a generalized stress response. PST is associated with complex hormonal changes that "prepare" the salmon for life at sea. It is a period of increased sensitivity as well (Carey and McCormick 1998). Post-smolt salmon typically grow rapidly in seawater (McCormick and Saunders 1987, McCormick *et al.* 1998). Slow or reduced growth may affect survival of salmon at sea (Scott 2001). Fairchild *et al.* (1999) speculated that exposure to 4-NP in a pesticide formulation may have contributed to the

poor return of adult salmon to New Brunswick, Canada rivers in subsequent years. Arsenault *et al.* 2004 showed that 4-NP and E2 affected growth hormone levels in the same group of fish assessed in our study. The change reduction in growth subsequent to exposure to 4-NP or E2 provides support for Fairchild's speculation that chemical exposure may have affected salmon returns to their native rivers.

The statistical approach described herein confirms the conclusions of Arsenault *et al.* (2004) and provides a method for assessing data from experiments conducted with other compounds. First of all, we determined functional dependences Time – Growth characteristics of smolts (linear for Time - Length and parabolic for Time - Weight) and evaluated statistical characteristics of these dependences. Secondly, we presented more details regarding growth: the location and size of time sub-segments where the difference between characteristics of fish without chemical treatment and under each type of chemical treatment was significant, not significant, or uncertain from statistical point of view. Such an approach may be useful in other cases for evaluation of the influence of environmental factors on dynamic systems.

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Appendix 1: List of Abbreviations

1. $L_{i,trng}(t)$ – mathematical models for dependences Time – Length constructed on the basis of training set
2. $W_{i,trng}(t)$ – mathematical models for dependences Time – Weight constructed on the basis of training set
3. $F_{obs\ trng}$ – observed values of F-criterion calculated for mathematical models on training set (Table 2)
4. $F_{obs\ val}(L_{i,trng}(t)$ – observed values of F-criterion calculated for $L_{i,trng}(t)$ on validation set
5. $F_{obs\ val}(W_{i,trng}(t))$ – observed values of F-criterion calculated for $W_{i,trng}(t)$ on validation set
6. $F_{obs\ val}$ – observed values of F-criterion calculated for mathematical models on validation set (Table 2)
7. F_{obs} – observed values of F-criterion calculated for mathematical models on the united set of observations (Table 3)
8. $F_{crit}(0.95, m, \infty)$, $F_{crit}(0.95, s, \infty)$ – values which were copied from F-distribution table